METABOLITES OF *STREPTOMYCES NOURSEI* III
INCORPORATION OF ^{14}C-CARBON DIOXIDE INTO CYCLOHEXIMIDE

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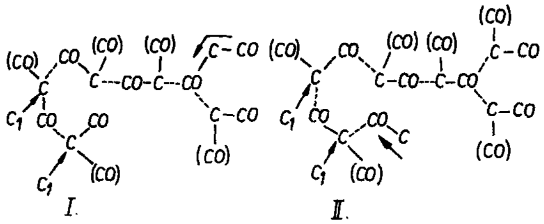
We have previously shown (Kharatyan *et al.*, 1963) that the biogenesis of cycloheximide (III) (Kornfeld *et al.*, 1949), and consequently that of related glutarimide antibiotics produced by *Streptomyces noursei*, *S.griseus* and *S.albulus*, involves methylation by C_1-donor agents (e.g., methionine) of a carbon skeleton derived fundamentally from acetate units. The polyketide skeleton of these compounds (e.g., III) could be derived in two main ways, represented diagrammatically as (I) and (II). In route (I), the chain would be initiated by malonyl- or malonamyl-coenzyme A [the latter is involved in oxytetracycline biosynthesis (Gatenbeck, 1961/62)] and elaborated by the addition of five more malonate units, which ultimately suffer decarboxylation. In the alternative route (II), the chain would be initiated by acetyl-coenzyme A and extended by six malonate units, oxidative loss of the terminal methyl group of this C_{16}-precursor then yielding the C_{15} cycloheximide-type skeleton.



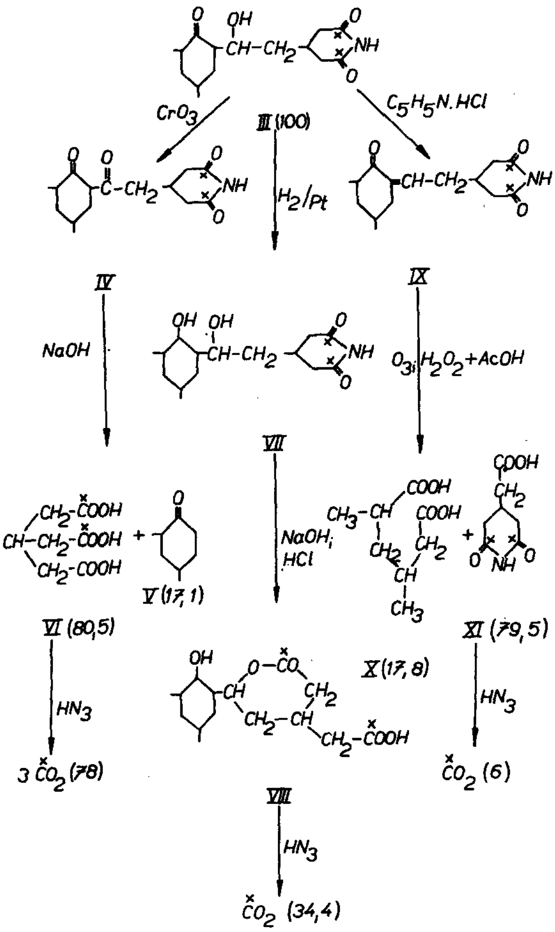
Our previous evidence (Kharatyan <u>et al</u>., 1963) favoured chain initiation by malonate or malonamate as in (I). We present here confirmation, derived from examination of the incorporation of ^{14}C-carbon dioxide into cycloheximide, of this biogenetic route to the glutarimides.

Experimental

500 ml. distillation flasks, each containing 80 ml. of medium (containing maize extract 0.5, soybean meal 2, glucose 8, ammonium sulphate 0.5, and calcium carbonate 0.5%) and maintained at 28° on a reciprocal shaker (98 r.p.m., amplitude 9.6 cm.), were inoculated with S.noursei (Institute of Microbiology strain 2/9, 48-hour mycelium grown under the same conditions). After 48 hours' growth, sodium ^{14}C-carbonate (200 μC) was distributed among 5 flasks. After fermentation for 120 hours, ^{14}C-cycloheximide was isolated by chloroform extraction, purified, and degraded as shown.

Results and Discussion

Oxidation of the ^{14}C-cycloheximide (III) to dehydrocycloheximide (IV), followed by alkaline hydrolysis as described by Kornfeld et al. (1949), gave relatively inactive 2,4-dimethylcyclohexanone (V) together with methanetriacetic acid (VI). The acid (VI) contained most (80.5%) of the original cycloheximide radioactivity, localised entirely in its carboxyl carbon atoms as shown by Schmidt degradation to carbon dioxide. Catalytic hydrogenation of cycloheximide (III) over platinum (Kornfeld et al., 1949) afforded dihydrocycloheximide (VII), which after alkaline hydrolysis followed by lactonisation with acid, yielded dihydrocycloheximidic acid (VIII). Schmidt degradation of this acid (VIII) gave carbon dioxide carrying 34.4% of the original cycloheximide activity, which, allowing for randomisation between the imide carboxyl groups during hydrolysis, corresponds to 68.8% of the overall activity residing in the imide carbonyl groups.



In order to confirm this labelling pattern, and in particular to confirm that the activity in the carboxyl groups of the tribasic acid (VI) originated from the imide ring rather than from the hydroxyl-carrying carbon atom in the antibiotic (III), anhydrocycloheximide (IX, prepared by dehydration with pyridine hydrochloride) was ozonised to yield a mixture of 2,4-dimethyladipic acid (X) and β-glutarimide acetic acid (XI). ^{14}C-Assay values for these compounds (X and XI) were in complete agreement with those of the previous fission products (V and VI, respectively), and decarboxylation of the acid (XI) gave carbon dioxide carrying insignificant radioactivity.

These results show that exogenous ^{14}C-carbon dioxide is being utilised for the carboxylation of acetyl-coenzyme A to malonyl-coenzyme A units. However, as the result of decarboxylation reactions following condensation of the malonate units, the ^{14}C-isotope is retained only in the case of the "primer" malonyl or malonamyl unit. This provides confirmation of biogenetic route (I) (Kharatyan _et al._, 1963) to the cycloheximide group, since if the alternative route (II), initiated by acetyl-coenzyme A, operated, no specific labelling of a glutarimide carbonyl group would be expected. The low activity distributed in the rest of the molecule is probably attributable to a slight equilibration of malonyl-coenzyme A with free malonic acid.

References

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